PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/415, C07D 233/84, 233/86, 233/72, 233/88, 405/04, 405/06

A1

(11) International Publication Number: **WO 97/00071**

(43) International Publication Date: 3 January 1997 (03.01.97)

(21) International Application Number: PCT/US96/10286

(22) International Filing Date: 13 June 1996 (13.06.96)

(30) Priority Data:
08/491,130 16 June 1995 (16.06.95) US

(71) Applicant *(for all designated States except US)*: BIOPHYSICA FOUNDATION [US/US]; 3333 North Torrey Pines Court #100, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SOVAK, Milos [DE/US]; 3333 North Torrey Pines Court #100, La Jolla, CA 92037 (US). BRESSI, Jerome, C. [US/US]; P.O. Box 126874, San Diego, CA 92112 (US). DOUGLASS, James, Gordon, III [US/US]; 4066 Moratalla Terrace, San Diego, CA 92130 (US). CAMPION, Brian [US/US]; 122 S. Rios Avenue #5, Solana Beach, CA 92075 (US). WRASIDLO, Wolfgang [US/US]; 307 Prospect Street, La Jolla, CA 92037 (US).

(74) Agents: ROWLAND, Bertram, I. et al.; Flehr, Hohbach, Test, Albritton & Herbert, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ANDROGENIC DIRECTED COMPOSITIONS

(57) Abstract

Substituted phenylthiohydantoins are provided for use in detecting the presence of tumor cells having androgenic receptors and providing for cytostatic and cytotoxic activity toward such cells. The subject compounds provide for vehicles for specific targeting to the endrogenic receptor containing cells of cytostatic and/or cytotoxic agents, heavy or light radioactive or radioopaque atoms, and the like for detection and treatment of cancer cells involving androgenic receptors or blocking androgenic receptors.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

ANDROGENIC DIRECTED COMPOSITIONS

INTRODUCTION

Technical Field

The field of this invention is diagnosis and treatment of androgenic related neoplasia and blockage of androgenic receptors.

Background

The growth of prostate cancer (CaP) depends upon the presence of androgen (male) hormones, acting via androgen receptors contained in the cell's nucleus. The only effective, albeit temporary, therapy of prostate cancer is based upon interference of male hormone production or activity, using estrogenic steroids or non-steroidal substances to block the cancer cells' androgen receptors. There are a number of problems with these therapies. Steroidal estrogens had to be abandoned due to their high cardiovascular toxicity. The only steroidal compound clinically used today is cyproterone acetate. However, it also binds to the glucocorticoid and progestin receptors. Current, clinically-used non-steroidal anti-androgens such as Flutamide, Casodex or Anandron do not bind sufficiently to androgen receptors to achieve their complete blockage. None of the current anti-androgens provide permanent relief. It is suspected that the incomplete blockage of the receptors may be the reason why, with time, the therapy invariably becomes ineffective as the CaP cells mutate having proliferated metastatically. At that phase, the cells cannot be substantially influenced by any known chemotherapy or radiation.

There is the further consideration that the current armamentarium for the diagnostic staging of prostate cancer is extremely poor and yet essential in choosing the therapeutic mode. Proof of metastatic dissemination beyond the prostate excludes surgery and relegates these patients to systemic therapy. With improved diagnostic staging, unnecessary prostatectomies, a major and potentially mutilating

surgery, could be avoided.

Only recently, an assay has become available for the detection of CaP cells circulating in the blood. However, that finding alone does not imply the existence of metastases. Typically, early metastases occur in the lymph nodes and the later ones develop in the bones. While ^{99}Tc scans can visualize bone defects, the lymph node metastases are extremely difficult to locate since typically, the infiltrated nodes are neither enlarged nor show changes on either magnetic resonance or x-ray computed tomography. Further, because of their low metabolic rate, the pathological nodes cannot be identified by positron emission spectrography using ^{18}F-deoxyglucose. Lymph node biopsy is possible only in the pelvic area. Early metastases in inaccessible paraaortic lymph nodes cannot be detected and consequently these patients are operated upon needlessly. Recently developed radiolabeled monoclonal antibodies against prostate cancer have only a limited use due to their low target specificity and long persistence in the blood pool, liver and spleen, which interferes with the imaging.

There have been a number of attempts to develop a CaP radionuclide scanning agent. Several radioiodinated androgen steroids were made, but they suffer from synthetic complexity. Steroidal androgens labeled with ^{18}F were synthesized as a potential PET imaging agent for prostate cancer, but their practicability is limited due to the complicated synthesis and need for specialized rare equipment (PET scanners) to detect positron emitting radionuclides. There is a further consideration that androgens promote CaP growth.

There is, therefore, substantial interest in developing novel compounds which can provide for the diagnosis and therapy of prostate cancer.

Relevant Literature

N-aryl substituted imidazolinediones have been reported in DE32 22 523; Offenlegungsschrift 26 49 925; WO88/03404; EP0 436426; EP0 494819; EP0 580459, and Teutsch, J. Steroid Biochem. Molec. Biol. (1994) 48:111-119. The activity of the trifluoromethyl, nitro- and trifluoromethyl, cyanophenyl derivatives as high-affinity ligands for the androgen receptor are reported in Teutsch, *supra.*, as well as in many of the foregoing patents.

SUMMARY OF THE INVENTION

Specific N-substituted 3-trifluoromethyl-4-cyano phenylthio-4',4'-dimethylhydantoins, their amino and thione analogs are provided having substitution at the remaining annular atom. Substituents include cyclic and aliphatic groups. Of particular interest are groups which can be used for imaging and/or have enhanced therapeutic index.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

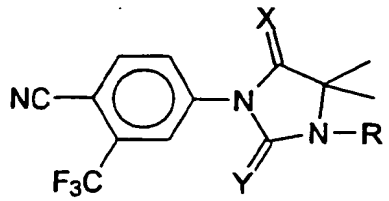
N-substituted arylthio-4',4'-dimethylhydantoins are provided, where when the 3'-N-substituent comprises other than an iodoaryl group, the hydantoin is a mono-thiohydantoin, where the other sp^2 carbon atom is bonded to oxygen, or nitrogen (imino). The compounds find use for diagnosis and/or therapy associated with androgenic receptors. The subject compounds have high affinity for androgen receptors of a variety of cell types and are able to exert at least one of proliferation inhibition or cytotoxicity for therapy or preferential binding for use as a detection medium for cells and tissues comprising androgenic receptors or for other identification.

For the most part, the subject compositions can be divided into three categories as characterized by the N-substituent: A group of from two to eight, usually from two to six carbon atoms, more usually from two to four carbon atoms, particularly two to three carbon atoms, which may be aliphatic or heterocyclic, generally having from zero to three, more usually from zero to two heteroatoms, preferably from one to two heteroatoms, which may be derivatized, particularly alkylated or acylated, where the alkyl or acyl group will be of from one to ten, more usually one to eight, preferably of from one to six carbon atoms, where the acyl group will generally be of from two to six carbon atoms, where the non-oxo-carbonyl may be bonded to from zero to two oxygen and/or nitrogen atoms, and zero to one carbon atoms; where the heterocycle will be from five to six annular members, particularly five annular members, where the annular members will be oxygen and nitrogen, generally having from 1 to 3 annular heteroatoms; the second group will have an agent, frequently a cytotoxic agent and/or imaging agent bonded to the hydantoin, normally through a linking group of from one to six, usually one to four carbon atoms, preferably two to three carbon atoms and one heteroatom,

where the linking group may include one or more functionalities, such as amino, oxy, and non-oxo-carbonyl, where amides and esters may be involved, e.g. urethanes; and the third group will involve carbocyclic aryl groups, particularly iodoaryl, which may be bonded to the nitrogen of the hydantoin through a linking group of from one to eight, usually two to six carbon atoms, preferably two to three carbon atoms, where the linking group may include an amino, oxy or non-oxo-carbonyl functionality, particularly ester or amide, and the aryl group may be substituted with oxy, amino, non-oxo-carbonyl, and derivatives thereof. As the aryl group, phenyl is of particular interest.

Tissue comprising cells with androgen receptors include prostate tissue, ovary tissue, testes, etc. Hosts of interest include primates, e.g. humans, domestic animals and pets.

The first group of the compounds of the subject invention will have the following formula:



wherein:

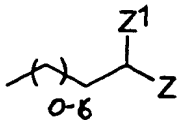
X is oxygen or nitrogen, with the proviso that when R is iodoaryl, X may be sulfur;

Y is sulphur, with the proviso that when R is iodoaryl group, Y may be sulphur, oxygen or nitrogen, preferably X and Y are different;

R is an organic group, which may be aliphatic and may comprise one or more heteroatoms, alicyclic, aromatic, heterocyclic, or combinations thereof, where heteroatoms include oxygen, nitrogen, sulphur etc., to be further defined below.

The first group of compounds will comprise monothiohydantoins, where the other oxo group of the hydantoin will be oxygen or nitrogen. These groups will, for

the most part, have R having the following formula:



wherein:

Z is hydroxyl, amino, a substituted amino or a 4-diazolyl, particularly a 4-(1',3'-imidazolyl);

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxolane.

The substituents on amino nitrogen may be varied widely, depending upon the use of the compound. For cytotoxicity or antiproliferative activity, the amino group may be unsubstituted or substituted, particularly with the single acyl group, where the acyl group may serve to enhance the activity of the compound by changing its pharmacokinetic activities, by providing for a second cytotoxic or antiproliferative compound, by providing for a chelating agent for chelating a metal ion, particularly a radioactive metal or non-metallic on, for carrying a radioopaque atom, or the like. Radioactive elements include fluorine, iodine, technetium, etc. Other metals of interest include gadolinium and the like.

Similarly, the hydroxyl, particularly the terminal hydroxyl, may be employed as a site for linking, forming ethers or esters, where the groups bound to oxygen will come within the above description; activations and displacement with other groups of interest, e.g. fluorine, sulphur alkyls and the like.

In addition, iodoaryl groups may be employed which are linked to the nitrogen through an alkyl chain, where the alkyl chain may be of from 1 to 6, usually from 1 to 4, preferably from 2 to 4 carbon atoms. The iodoaryl group may be linked directly to the carbon of the alkyl group or linked through a heteroatom, particularly nitrogen or oxygen, e.g. amide, secondary amine, ether, ester, etc. where the iodoaryl group may have a non-oxo-carbonyl or amino group linked to an annular carbon atom as part of the linking chain. The iodoaryl will generally have from 1 to 4, usually 2 to 4, more usually 2 to 3 iodine atoms, and may be further

substituted with oxy, particularly hydroxy or alkoxy of from 1 to 3 carbon atoms, or amino, or a substituted amino (mono- or disubstituted), having alkyl substituents having a total of 1 to 6 carbon atoms, more usually 1 to 4 carbon atoms, and 0 to n-1 oxy groups, where n is the number of carbon atoms in the substituent. A variety of aminosubstituted symmetrically substituted triiodoisophthaldiamides and diaminosubstituted symmetrically substituted triiodobenzamides have been reported in the literature, where the nitrogen atoms are substituted with acyl groups, alkyl groups or oxyalkyl groups of 1 to 6, usually 1 to 4 carbon atoms and 0 to n-1 oxy groups. See, for example, U.S. Patent Nos. 4,547,357; 4,021,481; 4,364,921 and 4,341,756 and references cited therein. The carboxyl group may be used to link the iodoaryl group to the thiohydantoin throgh the alkyl chain. Alternatively, iodine may be bonded to an sp_2 carbon atom of an alkenyl group.

Illustrative R groups include: allyl, propynyl, aminoethyl, aminopropyl, 2-hydroxypropyl, 3-hydroxypropyl, 2-hydroxyethyl, 2,3-dihydroxypropyl, 2-hydroxy-3-acetoxypropyl, 4-benzamidobutyl, 4-fluorobutyl, 4-iodobut-3-enyl, 2-iodoprop-2-enyl, cis & trans-3-iodo-prop-2-enyl, 3-(4'-oxazolyl-1,3)propyl, 2-(4'-diazolyl)ethyl, 3-(propionamido)propyl, N-phenoxycarbonyl 2-aminoethyl, N-methoxycarbonyl 2-aminoethyl, 3-(3',5'-diiodo-4'-dimethylaminophenyl)propyl, 2-(3',4',5'-triiodophenyl)propyl, N-(cysteinyl, glycyl, glycyl) 2-aminoethyl, (3',6',9'-triazanonoxy)ethyl, p-hydroxyphenylpropyl, and the carboxamide of N-nitrilotriacetic acid and 2-aminoethyl.

Alternatively, various cytotoxic agents may be employed, which are joined to the subject hydantoins by any convenient linking group, which does not significantly diminish the cytotoxic or antiproliferative activity of the compound. Compounds of interest include methotrexate, taxol, 5-fluorouracil, adriamycin, bleomycin, and the like.

The subject compounds can be prepared in accordance with conventional ways, varying the particular procedure based on the particular side groups. The preparation of hydantoins conveniently involves the use of an isocyanate and a substituted α-aminoacetonitrile. By appropriate choice of the isocyanate and the α-aminoacetonitrile, one may arrive at the final product in a single step. Alternatively, by employing various protective groups, which may be subsequently removed, or providing for substituents which become involved in the formation of

the hydantoin or may provide for sites for further derivatization. Various procedures are described in EPO Publication Numbers 0 494 819 and 0 580 459. Also, a significant number of examples may be found in the subject experimental section.

The subject compositions find a variety of uses associated with prophylactic and therapeutic opportunities. By providing for substituents which allow for detection by x-rays, molecular resonance imaging, radioactivity, or the like, regions of a mammalian host, particularly humans, can be investigated, where the regions are associated with an androgenic receptor. Thus, cells or tissues associated with the androgenic receptors may be visualized, so as to identify neoplasms, benign tumors, mobile cells, etc. Thus, by having substituents which have radioactive atoms, heavy metals, heavy atoms such as iodine, or the like, one can visualize physiological structures associated with androgenic receptors.

In addition, the subject compounds have proliferative inhibitory capability in inhibiting the proliferation of cells having androgenic receptors and dependent upon signal transduction associated with the androgenic receptors. The subject compounds are found to have a high affinity for the androgenic receptors, demonstrating enhanced activity as compared to prior substituted hydantoins.

In addition, the subject hydantoins can be used as vehicles for transporting other cytotoxic agents to the androgen receptor comprising cells. Thus, while at the same time inhibiting androgenic activation, other pathways which inhibit proliferation may also be addressed. Thus, one can greatly enhance the therapeutic index of a known chemotherapeutic agent by directing the chemotherapeutic agent to specific sites in the host.

The subject compositions may be formulated in accordance with conventional ways for use *in vivo*. The subject compounds are found to be stable in human plasma at physiological temperatures. The subject compounds are found to have substantially greater cytostatic and cytotoxic effects in inhibiting cell growth for neoplastic cells, as compared to normal cells, i.e. having a high therapeutic index. The subject compositions are readily formulated in conventional carriers, such as saline, phosphate buffered saline, vegetable oils, ethanol, or other physiologically acceptable carrier.

The concentrations used for the subject compounds in diagnosis and therapy

will be varied widely, depending upon the purpose of the compound, the patient being treated, the stage of the disease, whether the subject compounds are being used by themselves or in a combination therapy, the manner of administration, the responsiveness of the cancer cells to the drug, and the like. The particular dosage can be determined empirically. Other components of the formulation may include buffers, stabilizers, excipients, or the like. Depending upon the particular compound and its formulation, administration may be oral or parenteral, including intravascular, subcutaneous, intratumoral, intraperitoneally, etc.

The subject compounds may also be used in competitive assays for evaluating other compounds as to their cytotoxic or cytostatic effect. Thus, specific cell lines may be employed where the effect of an agent on the cytotoxic level of a subject compound may be determined in relation to the survival rate of the target cells. Also, in mixtures of cells containing neoplastic androgenic receptor containing cells the subject compounds can be used to eliminate the neoplastic cells in the presence of normal cells. Thus, in a variety of cultures, where androgenic receptor containing cells may be susceptible to becoming or are tumorous, by maintaining a cytoxic level of the subject compounds in the medium, the cells may be selectively killed.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

The following compounds were prepared according to the general method described by Teutsch et. al., J. Steroid Biochem. Molec. Biol. 1994; 1:11-119.

Example 1

4-[3′-(2″-(N-t-butoxycarbonyl)-aminoethyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-136)

Crude 2-trifluoromethyl-4-isothiocyanoato-benzonitrile (700 mg, 3.07 mmol) was dissolved in THF (6.0 mL). At room temperature, triethylamine (59μL, 0.42 mmol) was added to the stirring solution followed by 2-(1′,2′-ethyldiamino-N-t-butoxycarbonyl)-2-cyanopropane (682 mg, 3.00 mmol). The reaction was refluxed for 40 minutes under a N_2 atmosphere and then the solvent was removed under

reduced pressure. The resulting brown residue was purified by silica gel chromatography (CH_2Cl_2/acetone, gradient) and treated with activated carbon to yield 951 mg (68.1%) of light yellow powder.

mp: 81°C (dec); UV (MeOH):λ_{max}=234 nm (ϵ=18841) and 260 nm (ϵ=21454)

Example 2

4-[3′-(2″,2″-dimethyl-1″,3″-dioxolane-4″-methyl)-4′,4′-dime thyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-163)

BP-163 was prepared and purified as described in Example 1 using the amino cyanopropane prepared from 2,2-dimethyl-1,3-dioxolane-4-methanamine and acetone cyanohydrin. Yield = 63.3%.

UV (MeOH): λ_{max}=230 nm (ϵ=23528), 244 nm (ϵ=22733), and 258 nm (ϵ=24590);

Example 3

4-[3′-(2″-propenyl)-4′,4′-dimethyl-5′′-imino-2′-thioxo-1′′-imidazolidinyl]-2-trifluoromethy l-benzonitrile. (BP-208)

BP-208 was prepared and purified by the same method described in Example 1 using the amino cyanopropane prepared from allylamine and acetone cyanohydrin. Yield = 67.3%.

Example 4

4-[3′-(2″-propynyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-im idazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-211)

BP-211 was prepared as described in Example 1 using the amino cyanopropane prepared from propargyl amine and acetone cyanohydrin. The compound was purified by chromatography (CH_2Cl_2/acetone, 100% (50:50 gradient by 10% segments) and isolated as an orange oil. The product was not further characterized and was carried as is into the hydrolysis step. (Example 12)

Example 5

4-[3′-(2″-{4′″-imidazolyl}ethyl)-4′,4′-dimethyl-5′-imino-2′ -thioxo-1′-

imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-210)

BP-210 is prepared as described in Example 1 using the amino cyanopropane prepared from 4-(aminoethyl)imidazole and acetone cyanohydrin. The compound is purified by column chromatography and isolated as a pale yellow oil. It is used in the subsequent hydrolysis without further purification. (Example 13)

Example 6

4-[3′-(2″-p-hydroxyphenylethyl)-4′,4′-dimethyl-5′-imino-2′- thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-212)

BP-212 was prepared as described in Example 1 using the amino cyanopropane prepared from p-hydroxyphenethylamine and acetone cyanohydrin. Following silica gel chromatography (CH_2Cl_2)/acetone; gradient), a pale yellow solid was obtained, which was taken directly into the hydrolysis step without further characterization. (Example 14)

Example 7

4-[3′-(2″-aminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-138)

BP-136 (300 mg, 0.66 mmol) was dissolved in MeOH (3.5 mL) and 2N HCl (.065 mL) with stirring at room temperature. The reaction mixture was refluxed for two hours, then the solvent was removed under reduced pressure, and the resulting solid was crystallized as the hydrochloride from isopropanol. Yield 204 mg (79.0%).

mp: >200(C; UV (MeOH): δmax =234 nm (18441) and 252 nm (ϵ=20891)

Example 8

4-[3′-(2″,3″-dihydroxypropyl)-4′,4′-dimethyl-5′-oxo-2′-thio xo-1′′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-135)

BP-135 was prepared in the manner described in Example 7 using the appropriate imine (BP-163, example 2). The product was isolated by pouring the reaction mixture over a mixture of ice and water. The product was extracted with EtOAc, dried over MgSO4 and the solvent removed under reduced pressure. BP-

135 was purified by silica gel chromatography (CH2Cl2/ acetone; gradient) then treated with activated carbon to yield a hygroscopic amorphous solid. Yield = 68.1%.

UV (MeOH): λ_{max}=234 nm (ϵ=17480) and 254 nm (ϵ=19963);

Example 9

4-[3'-(2"-propenyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1''-imid azolidinyl]-2-trifluoromethyl-benzonitrile. (BP-82)

BP-82 was prepared in the same manner as described in Example 7 using the appropriate imine (BP-208, example 3). The product was isolated by pouring the reaction mixture over a mixture of ice and water. The product was extracted with EtOAc, dried over MgSO4 and the solvent removed under reduced pressure. BP-82 was purified by treatment with activated carbon and crystallization from isopropanol. Yield = 87.4%.

mp: 146-148°C; UV (MeOH):λ_{max}=232 nm (ϵ=18022) and 254 nm (ϵ=21877)

Example 10

4-[3'-(2"-N-(t-butoxycarbonyl)-aminoethyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-137)

BP-137 was prepared from BP-136 in the same manner as described in Example 7 except the reaction was heated at 50°C for eight hours. The resulting white crystalline precipitate was filtered off and washed with cold MeOH/H_2O (50:50). Yield = 87.1%.

mp: 173-175°C; UV (MeOH): λ_{max}=234 nm (ϵ=18573) and 256 nm (ϵ=21499)

Example 11

4-[3'-[2",2"-dimethyl-1",3"-dioxolane-4"-methyl)-4',4'-dime thyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benz onitrile. (BP-134)

BP-134 was isolated as an impurity in the silica gel chromatographic purification of BP-163.

mp: 50°C (dec); UV (MeOH): λ_{max}=234 nm (ϵ=18765) and

254 nm (ϵ=21499)

Example 12

4-[3'-(2"-propynyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-199)

BP-199 was prepared from the appropriate imine (BP-211, example 4) in the same manner as described in Example 7. The product was isolated as colorless crystals from CH_2Cl_2/hexane.

mp: 120-121°C (dec); UV:λ_{max}=206 nm (ϵ=17328), 232 nm (ϵ=18068), and 252 nm (ϵ=22003).

Example 13

4-[3'-(2"-{4"'-imidazolyl}ethyl)-4',4'-dimethyl-5'-oxo-2'-t hioxo-1'-imidazolidinyl-2-trifluoromethyl-benzonitrile. (BP-213)

BP-213 was prepared from the appropriate imine (BP-210, example 5) in the same manner as described in example 7. The crude product was purified by column chromatography and isolated as a colorless solid in high purity ((96%, HPLC).

UV: λ_{max}=234 nm (ϵ=14113) and 254 nm (ϵ=1604).

Example 14

4-[3'-(2"-p-hydroxyphenylethyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-214)

BP-214 is prepared from the corresponding imine (BP-212, example 6) in the same manner as described in Example 7. The crude product is crystallized from CH_2Cl_2/hexane as colorless crystals.

Example 15

4-[3'-(2"-N-acetylaminoethyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-139)

The free amine of BP-138 (100 mg, 0.28 mmol) was dissolved in $(Ac)_2O$ (5.0 mL) and allowed to stir at room temperature for 30 minutes. The solvent was then removed under reduced pressure and the resulting off-white solid was purified by silica gel chromatography (CH_2Cl_2/acetone; 95:5) to yield 102 mg (91.6%) of

pure compound.

mp: 77-79°C (dec); UV (MeOH):λ_{max}=234 nm (ϵ=18694) and 254 nm (ϵ=21499)

Example 16

4-[3′-(2″-aminoethyl-N-(glycyl-N′″-(2′″-(triphenylmethylthioacetyl)-glycine)))-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-197)

Dicyclohexylcarbodiimide (DCC, 1.1 mg, 5.35 x 10-3 mmol) and the free base of BP-138 (1.9 mg; 5.35 x 10-3 mmol) were added to a stirring solution of N-[2-triphenylmethylthioacetyl)]-glycyl-glycine (2.0 mg, 4.46 x 10-3 mmol) in THF (0.200 mL) at room temperature. The reaction was heated at 35°C for two hours and then purified by preparative HPLC without further work-up. Yield = 50.2%.

Example 17

4-[3′-(4″-oxybutyl-O-glycyl-N′″-(2-(triphenylmethylthioacetyl)-glycine))-4′,4′-dimethyl- 5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-198)

To a stirred solution of N-[2-(triphenylmethylthioacetyl)]-glycyl-glycine (2.0 mg, 4.46 x 10-3 mmol) in THF (2.00 mL) was added DCC (1.1 mg, 5.35 x 10-3 mmol),4-[3'-(4"-hydroxybutyl)-4',4'-dimethyl-5'-oxo-2'-thioxo -1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile (RU 59063, 2.1 mg, 5.35 x 10-3 mmol) [Synthesized as described by Teutsch et. al., supra] and a crystal of DMAP. After stirring at room temperature for 45 minutes, the product was isolated by preparative HPLC. Yield = 56.8%.

Example 18

4-[3′-(2″-aminoethyl-N-(glycyl-N′″-(2-thioacetyl)-glycine) -4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-207)

Bu_3SiH is added to a stirring solution of BP-197 in 10% TFA/CH_2Cl_2 and is purified by preparative HPLC without further work-up. This product can now be used as a substrate for complexing with 99Tc by standard methods.

Example 19

4-[3'-(4"-oxybutyl-O-glycyl-N'"-(2-(thioacetyl)-glycine))- 4',4'-dimethyl-5'-oxo-2''-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-209)

Bu_3SiH is added to a stirring solution of BP-198 in 10% TFA/CH_2Cl_2 and is purified by preparative HPLC without further work-up. This product can now be used as a substrate for complexing with ^{99}Tc by standard methods.

Example 20

4-[3'-trans-(2"-propenyl-3"-tributylstannyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-237)

BP-199 (1.05 g) was dissolved in dry toluene (100 mL) under N2. Bu3SnH (1.12 mL) and AIBN (68.5 mg) were added and the reaction mixture heated to reflux. After stirring for 24 hours, additional aliquots of Bu_3SnH (0.40 mL) and AIBN (10 mg) were added. After further stirring for 3 hours at reflux, the reaction was allowed to cool to room temperature and the volatiles removed under vacuum. The crude product was purified by column chromatog raphy and isolated as a pale oil (1.67 g).

HPLC analysis indicated the presence of two isomers.

Example 21

4-[3'-trans-(2"-propenyl-3"-*iodo)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl-2-trifluoromethyl-benzonitrile. (BP-305)

BP-237 is dissolved in a small amount of methanol. Radioiodination is accomplished using Na[^{125}I]I or Na[^{131}I]I or Na[^{123}I]I by known methods (see Hunter & Greenwood, Nature, 1962; 194:495-496]. TLC with autoradiography indicates 50-75% radiochemical yield.

Example 22

4-[3'-(4"-methanesulfonyloxybutyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-232)

RU-59063 (described by Teutsch *et al.*, supra and Example 17), was dissolved in methylene chloride, pyridine was added and the solution cooled to 0°C. Under N_2, methanesulfonic anhydride was added slowly and the reaction allowed to

warm to room temperature. The solution was cooled and pyridinium hydrochloride is filtered. The product was purified column chromatography (silica gel, $CHCl_3$/acetone; gradient 100% (85:15) and isolated as a colorless solid. m.p. 114-115°C.

Example 23

4-[3'-(4"-fluorobutyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-218)

a.) ^{19}F BP-218

RU 59063 (2.2 g) was placed in a 100 mL Schlenk flask with a stir bar and placed under N_2. Dry methylene chloride was added (15 mL) and the solution stirred under N_2 for 10 minutes. Pyridine (1.66 mL) was added, the solution cooled to -78°C with dry ice/acetone bath. Dimethyl aminosulphur trifluoride (DAST, 0.905 mL) was added dropwise and the reaction stirred at -78°C for 4 hours. The solution was then allowed to warm to room temperature and then taken to dryness. The product was isolated as a colorless oil by column chromatography (260 mg).

b.) ^{18}F BP-218

[^{18}F] Fluoride ion was produced by proton irradiation of oxygen-18 enriched (96% isotopic enrichment) held in an all-silver cyclotron target (330 μL target volume). The aqueous [^{18}F] fluoride was converted to a no-carrier added Kryptofix 2.2.2/K_2CO_3/^{18}F; prepared by addition of the [^{18}O] water/[^{18}F] solution to a mixture of the aminopolyether 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane (Kryptofix 2.2.2, 26.0 mg, 0.069 mmole) and potassium carbonate (2.3 mg, 0.0166 mmole) in a Vacutainer®. The vessel was placed in an oil bath at 110°C, and water was removed under a gentle stream of N_2, assisted by azeotropic distillation, each employing 0.5-0.8 mL CH_3CN.

The Kryptofix/K_2CO_3/^{18}F solution (1-50 mCi) in anhydrous acetonitrile (500 (L) was added to 2.0 mg of (4-[3'-(4"-methanesulfonyloxybutyl)-4'-4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl-2-trifluoromethyl-benzonitrile) (BP-232). The reaction mixture was heated for one hour at 110°C and then cooled before being injected onto a preparative HPLC system. The HPLC purification was performed on a C-18 reverse-phase preparative column and eluted with a 65:35 CH_3CN/H_2O solvent mixture (2 mL/min). Column effluent was monitored by a flow-through

radiation detector at 254 nm. The desired F-18 compound eluted at ~ 19 minutes. The solvents were evaporated in vacuo and the ^{18}F BP-218 was reformulated in saline.

Both radio-HPLC and radio-TLC were used to determine radiochemical purity. Purity by HPLC was determined using an ODS reverse-phase column, eluting with acetonitrile/water (80/20) with UV detection at 254 nm and a flow-through radiation detector. The retention time for F-18 BP-218 was 6.2 min.

Radio-TLC were performed as follows: silica gel plates; $CHCl_3$/acetone (95:5); F-18 BP-218 (Rf=0.5).

Example 24

7{5",5"-dimethyl-4"-oxo-3"-[4"'-cyano-3'"-trifluoromethylphenyl-1'-imidazolidinyl]-2"-thioxo-1"-ethylcarbamoxy}paclitaxel. (BP-196)

A round bottom flask charged with paclitaxel (60 mg, 0.07 mmol), imidazole (90 mg, 1.32 mmol) and a magnetic stir bar was placed under a N_2 atmosphere. CH_2Cl_2 (1.5 mL) was added and the solution was stirred at room temperature. To the solution was added portionwise a solution of 1.0 M $ClSiEt_3$ in THF (5 x 100 μL, 0.5 mmol). The progress of the reaction was monitored by HPLC. Upon completion, the 2'-(triethylsiloxy)paclitaxel was purified by preparative HPLC yielding 51.3 mg (75%). Purity by HPLC 97%. Proton NMR of the product matched values given in the literature [Chandhary et. al., J. Org. Chem. 1993; 58(15):3798-3799]

A round bottom flask charged with 2'-(triethylsiloxy)paclitaxel (30 mg, 0.03 mmol) and p-nitrophenylchloroformate (310 mg, 1.50 mmol) and a magnetic stir bar was placed under a N_2 atmosphere. A solution of pyridine (200 μL, 0.247 mmol) in CH_3CN (1.0 mL) was added and the mixture stirred at room temperature for 30 minutes. The product 2'-(triethylsiloxy), 7-(p-nitrophenylcarbonoxy)paclitaxel was purified by preparative HPLC yielding 24.2 mg (69%). Purity by HPLC was 96%.

To a round bottom flask charged with 2'-(triethylsiloxy), 7-(p-nitrophenyl-carbonoxy)paclitaxel (28.0 mg, 0.014 mmol), 4-[3'-(2"-aminoethyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile (2 X 8.0 mg, 0.44 mmol) and a magnetic stir bar was added CH_2Cl_2 (300 μL). The solution was stirred at room temperature for 4 hours and the product, 2'-(triethylsiloxy)-7{5",5"-

dimethyl-4″-oxo-3″-[4′″-cyano -3′″-trifluoromethylphenyl-1′-imidazolidinyl]-2″-thioxo-1″-ethylcarbamoxy}paclitaxel, was purified by preparative HPLC yielding 8.2 mg (85%). Purity by HPLC 97%.

To a round bottom flask charged with 2′-(triethylsiloxy)-7{5″,5″-dimethyl-4″-oxo-3″-[4′″-cyano-3′″- trifluoromethylphenyl-1′-imidazolidinyl]-2″-thioxo-1″-ethylcarb amoxy}paclitaxel (5.0 mg, 0.004 mmol) and a stir bar was added formic acid (250μL). The solution was stirred at room temperature for 15 minutes and the volatiles removed under vacuum. BP-196 was purified by preparative HPLC yielding 4.6 mg (>99%). The purity by HPLC was 99%.

Example 25

4-[3′-(2″-{4″′-(2″′*iodo)imidazoyl}ethyl)-4′,4′-dimethyl-5′ -oxo-2′-thioxo-1′-imidazolidnyl]-2-trifluoromethyl-benzonitrile . (BP-216)

BP-213 is dissolved in methanol. Radioiodination is accomplished with chloramine-T and Na[^{125}I]I or Na [^{131}I]I or Na [^{123}I]I by standard methods [Hunter and Greenwood, Nature, 1962; 194: 495-496] The product is purified by HPLC.

Example 26

4-[3′-gem-(2″-propenyl-2″-tributylstannyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl)-2-trifluoromethyl-benzonitrile. (BP-300)

BP-199 (2.30 g) was placed in a three-neck 500 mL round bottom flask fitted with two rubber septa, an N_2 adapter and stir bar. Dry toluene was added (30 mL) followed by HSnBu3 (2.48 g). $Pd(PPh_3)_4$ (151 mg) was dissolved in toluene (30 mL) and added quickly to the previously prepared solution. After 24 hours of stirring at room temperature, an additional aliquot of $Pd(PPh_3)_4$ (50 mg) was added and the reaction heated at 65°C for 3 hours followed by stirring at room temperature for 48 hours. The reaction mixture was taken to dryness and the product(s) purified by column chromatography. HPLC analysis (C18 reverse phase, 75:25 ACN/H_2O) suggested that the major product was BP-300 and the minor product was BP-237 (79:21), based on NMR comparison of the corresponding iodo compounds. (Example 29)

Example 27

4-[3'-trans--(2"-propenyl-3"-iodo)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-305)

and

4-[3'-cis--(2"-propenyl-3"-iodo)-4',4'-dimethyl-5'-oxo-2'-t hioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-307)

BP-237 (82% pure, with the remainder the corresponding cis isomer BP-354, 370 mg) was dissolved in $CHCl_3$ (5 mL) and cooled to 0°C. In a separate flask I_2 was dissolved (146 mg) in $CHCl_3$ (15 mL) and added to the solution of BP-237. After 2 hours at room temperature, the volatiles were removed and the crude products separated and purified using column chromatography.

Example 28

4-[3'-(6"-methanesulfonyloxyhexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-328)

BP-327 (10.4g) was dissolved in methylene chloride (130 mL), pyridine (2.5 mL) was added and the solution was cooled to 0°C under N_2. Methanesulfonic anhydride (5.5 g) was dissolved in methylene chloride (100 mL) and the resulting clear solution added slowly to the former solution. After 30 minutes at 0°C, the solution was allowed to warm to room temperature at which time the volatiles were removed under vacuum. The crude product was dissolved in a minimum of chloroform, filtered, and purified using silica gel column chromatography. Combining the appropriate fractions followed by removal of volatiles gave the product as a light brown oil (8.8 g, 98% pure by HPLC).

Example 29

4-[3'-(6"-thiohexyl)hexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo- 1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-332)

BP-328 (1.10 g) was dissolved in methylene chloride (35 mL). In a separate flask were placed hexanethiol (315 μL) and toluene (10 mL). Sodium methoxide (403 μL, 5.5 M) was added and the solution stirred for ten minutes. The resulting emulsion was added dropwise to the BP-328 solution with rapid stirring. After stirring for 12 h, the solution was stripped down and the crude product was purified

by column chromatography and isolated as a clear oil (160 mg) in 25% recovered yield. Additionally, unreacted BP-328 was also recovered (50%).

Example 30

4-[3′-{2″-N-(p-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl benzonitrile. (BP-231)

A 100 mL Schlenk flask was charged with BP-138 (430 mg, 1.20 mmol), Bolton-Hunter reagent (318 mg, 1.20 mmol) and a stir bar. Anhydrous THF (5 mL) was added via a gas tight syringe and the reaction mixture stirred under N_2(g) at room temperature. After one hour, the volatiles were removed under vacuum and the crude product purified using column chromatography (230-400 mesh SiO_2, 20 g, packed with $CHCl_3$) using gradient elution (100% CHCl3 (80:20 $CHCl_3$/Acetone). The appropriate fractions (as determined by TLC) were combined and the volatiles removed to give the product as a white solid (385 mg) in 64% yield. The purity by HPLC was 99.0%. UV (MeOH): λ_{max}=206 nm (ϵ=9553), 228 nm (ϵ=9872), 254 nm (ϵ=8339).

Example 31

4-[3′-{2″-(N-3‴,5‴-diiodo-4‴-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl-benzonitrile. (BP-248)

BP-231 (54.2 mg, 0.107 mmol) and chloramine-T (60 mg) was placed in a round bottom flask and $CHCl_3$ (6 mL) added. Iodine was added (6.05 mg). Methanol (3 mL) was added dropwise at room temperature with stirring. The solution turned orange. After one hour, the reaction was quenched ($Na_2S_2O_5$ 50 mg in 5 mL H_2O) and the products extracted into $CHCl_3$ (2 x 10 mL). The combined organics were dried and the volatiles removed. The crude product was purified using column chromatography (SiO_2, 5 g, $CHCl_3$) with a gradient elution (100 $CHCl_3$ (95:5 $CHCl_3$/Acetone). Purity was 97% based on HPLC. Mass Spec: MH+ (757).

Example 32

4-[3'-(6"-hydroxyhexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-327)

The amino cyanopropane derived from 6-hydroxyhexyl amine and acetone cyanohydrin (13.9 g, 75.7 mmol) was dissolved in THF (100 mL). In a separate flask was placed the substituted aryl isothiocyanate (17.2 g, 75.7 mmol) to which was added THF (50 mL) and NEt_3 (2.0 mL). The latter orange solution was added to the former with stirring. After 12 hours, the volatiles were removed under vacuum to give the crude imine cyclization product as an viscous orange oil. This product was dissolved in methanol (350 mL) and subjected to HCl (2N, 94 mL, 0.187 mmol). Heat evolved. After 30 minutes, the volatiles were removed under vacuum. The product was purified using column chromatography (250 g, SiO2, $CHCl_3$) and a gradient elution (100 CHCl3 (80:20 $CHCl_3$/Acetone). 26.0 g of product was obtained (light brown oil). Purity by HPLC: 98.8%.

Testing:

All compounds were tested for stability by incubation in human plasma at 38°C for three hours and subsequent analysis by high pressure liquid chromatography. All compounds tested were found to be stable under these conditions.

All compounds were screened on a panel of normal and cancer human cell lines, including human prostate cancer cell lines, PC-3, DU-145, and LnCAP. The purpose of this experiment was to assess cell growth inhibition by measuring cytotoxicity and cytostatic effects.

Cells (10^4/well) were plated on 96 well plates with the following controls: no cells and toxic control (1 x 10^{-3}M sodium dodecyl sulfate (SDS). The drug was diluted in ethanol and added directly to the wells. Plates were incubated at 37°C under 5% carbon dioxide in sterile air, in a humidified incubator for 72 hours. A solution (50 μl of 2,3-bis-(methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT), 1 mg/mL) in phosphate buffered saline (PBS, 100 mM) was added to each well, In the presence of viable cells, this colorless clear solution is enzymatically transformed to give a pink coloration, read at 450 nm using a microplate reader (Molecular Devices Thermomax). The inhibition of cell growth was measured by hemocytometer,

counting cell viability. (Table I)

The results of compounds hitherto investigated are shown in Tables I and II. While the cytostatic effect of BP-82 is demonstrated in PC-3 human cell line (Table II), the growth inhibition (which reflects primarily cytotoxicity and may obscure the cytostatic property) is shown for compounds BP-196 and BP-199.

It is not certain whether the cytoxicity of BP-196 can be ascribed to the taxol moiety. The toxicity of this compound vis-a-vis normal cells is also quite high.

On the other hand, it appears that such targeting does occur with BP-199 which is most cytotoxic in the human prostate cancer lines containing at least some androgen receptors, but has low cytotoxicity in a variety of other human transformed and normal cells.

The androgenic and anti-androgenic activity of the current and novel compounds was tested in a specific assay described by Fuhrman et al. [J. Steroid Biochem. Molec. Biol. 1992;42:787-793]. This assay uses CV-1 cells derived from monkeys transfected with human androgen receptors. (Table III and IV).

TABLE I

Inhibition of Cell Proliferation at 72 hours:
Cytotoxic Effects of the Selected Novel Anti-Androgens.

		IC_{50} [M]		
Cell Line	Tumor	BP-82	BP-196	BP-199
DU-145	Human Prostate (receptor poor)	1.39×10^{-5}	8.67×10^{-7}	8.51×10^{-5}
Ln CAP	Human Prostate (with androgen receptors)	6.60×10^{-5}	1.31×10^{-7}	8.20×10^{-7}
PC-3	Human Prostate (few androgen receptors)	3.15×10^{-5}	3.72×10^{-8}	1.32×10^{-7}
MCF-7	Human Breast	5.00×10^{-5}	9.89×10^{-7}	1.00×10^{-4}
MCF-7/ADR	Human Breast (adriamycin resistant)	1.51×10^{-5}	1.00×10^{-5}	1.00×10^{-5}
Ovcar 3	Human Ovary	9.65×10^{-5}	5.00×10^{-8}	$> 10^{-4}$
Molt-4	Human T-cell Leukemia	4.88×10^{-5}	1.47×10^{-7}	$> 10^{4}$
L-1210	Mouse Leukemia	2.50×10^{-5}	9.70×10^{-7}	1.10×10^{5}
	Normal			
NH DF	Dermal Fibroblast (human)	9.17×10^{-5}	1.07×10^{-7}	$>10^{-4}$
HLF-1	Normal Lung Diploid (human)	3.90×10^{-5}	8.06×10^{-6}	$>10^{-4}$
CHO	Chinese Hamster Ovary	3.45×10^{-5}	8.76×10^{-6}	1.28×10^{-5}

TABLE II

Relative Growth Inhibition*
Hydantoin Derivatives at 10^{-5} M after 6 days.

Compound	No. of cells remaining expressed as a % of control	Observation
BP-82	≈70%	growth reduction only
BP-196	≈100%	cytotoxic cell death
BP-199	≈50%	growth reduction only
BP-213	≈40%	some cytotoxicity and growth reduction
BP-231	≈30%	growth reduction only

*Cell density 10^4/well

TABLE III

Anti-androgenic potency (IC_{50}) of current and novel anti-androgens.

Transactivation assay in CV1-3.9.2 cells; Stimulation with 0.1 nM testosterone)

COMPOUND	IC_{50}[nM]
Cyproterone Acetate	11
RU59063†	23
Hydroxyflutamide	35 (Binding Affinity [Kf]*=280
Casodex	180
BP134	21
BP135	158
BP136	200
BP137	20
BP138	139
BP139	239
BP199	15 (Binding Affinity [Kf]*=5
BP82	≈ 6.5
BP163	217
BP 307	7 (Binding Affinity [Kf]*=24
BP 305	100 (Binding Affinity [Kf]*=15
BP 306	10 (Binding Affinity [Kf]*=23
BP 82	~6.5 (Binding Affinity [Kf]*=28
BP 231	260 (Binding Affinity [Kf]*=56
BP 328	NA (Binding Affinity [Kf]*=52
BP 218	NA
BP 332	NA

* Kf=competition factor, Kf=1=same as R1881
†Described by Teutsch, (Ref. 1)

TABLE IV

Androgen Activity of Anti-Androgens in CVI-3.9.2 Cells

Test Compounds*	CAT Activity [cpm]
EtOH+	2250
R1881 (0.1nM)+	5400
R1881 (1.0nM)+	5600
R1881 (10nM)+	6700
RU59063	2600
BP134	1600
BP135	1900
BP136	1800
BP137	2000
BP138	1600
BP139	1500
BP82	1300
BP163	2100

* (Except as indicated, all compounds were tested at 1 μM)
+ Controls

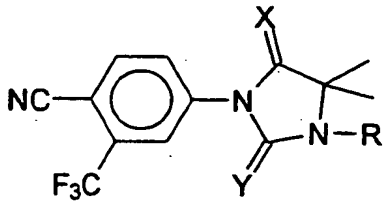
It is evident from the above results, that the subject compounds provide for a variety of advantages in directing a variety of agents to androgenic receptors of cells. Substantial therapeutic index is available between tumor cells and normal cells. The compounds are stable and can be readily formulated in a variety of ways. In addition, the subject compounds can be used as vehicles for bringing to tumor cells having androgenic receptors, cytotoxic agents, contrast agents, radioactive atoms, and the like. In this way, tumors having androgenic receptors may be visualized, as well as treated therapeutically.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound of the formula:



wherein:

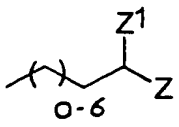
X is oxygen or nitrogen, where the proviso that when R is amino, oxy or iodo substituted aryl, X is sulfur, oxygen or nitrogen;

Y is sulphur, with the proviso that when R is said aryl group, Y is sulphur, oxygen or nitrogen;

R is an organic group comprising an aliphatic linking group of from 0 to 2 oxy groups, 0 to 1 amino group, 0 to 1 halo group, or 0 to 1 imidazolyl group, wherein said oxy groups, said amino group and said imidazolyl group have from 0 to 1 substituent.

2. A compound according to Claim 1, wherein R comprises an annular ring amino or oxy substituted aralkyl group for iodination or a polyiodoaralkyl group, wherein said aryl portion is linked to said alkyl portion by a carbon-carbon bond or through a heteroatom.

3. A compound according to Claim 1, wherein R is of the formula:



wherein:

Z is hydroxyl, amino, substituted amino, halo or 4-diazolyl;

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxalane.

4. A compound according to Claim 3, wherein Z and Z^1 are taken together.

5. A compound according to Claim 3, wherein Z is hydroxyl.

6. A compound according to Claim 3, wherein Z is amino.

7. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent being acyl or alkyl of from one to ten carbon atoms.

8. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent is a chelating group.

9. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent is an antibiotic.

10. A compound according to Claim 9, wherein said antibiotic is paclitaxel.

11. A compound according to Claim 3, wherein Z is a substituted amino group, wherein the substituent of said substituted amino group is a polyiodoaryl group.

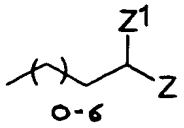
12. In a method for specifically directing an agent to cells comprising an androgenic receptor by adding said agent to a mammalian host comprising said cells, the improvement which comprises:

said agent being a compound according to Claim 1.

13. A method according to Claim 12, wherein said substituent is an antibiotic.

14. A method according to Claim 12, wherein said substituent comprises a radioactive atom or heavy atom.

15. A method according to Claim 1, wherein R is of the formula:



wherein:

Z is hydroxyl, amino, halo or 4-diazolyl;

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxalane.

16. A compound selected from the group consisting of: 4-[3′-(2″-propenyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′′-imid azolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-(N-t-butoxycarbonyl)-aminoethyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-N-acetylaminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-propynyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-trans-(2″-propenyl-3″-*iodo)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl-2-trifluoromethyl-benzonitrile; 4-[3′-cis--(2″-propenyl-3″-iodo)-4′,4′-dimethyl-5′-oxo-2′-t hioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(6″-thiohexyl)hexyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo- 1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-{4″′-(2″′*iodo)imidazoyl}ethyl)-4′,4′-dimethyl-5′ -oxo-2′-thioxo-1′-imidazolidnyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(4″-fluorobutyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-trans-(2″-propenyl-3″-tributylstannyl)-4′,4′-

dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-gem-(2″-propenyl-2″-tributylstannyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl)-2-trifluoromethyl-benzonitrile; 4-[3′-{2″-N-(p-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl benzonitrile; 4-[3′-{2″-(N-3‴′,5‴′-diiodo-4‴′-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl-benzonitrile; and 4-[3′-(6″-methanesulfonyloxyhexyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10286

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/415; C07D 233/84, 233/86, 233/72, 233/88, 405/04, 405/06.
US CL :514/386, 342, 391; 548/311.1, 317.1, 318.5, 320.1, 320.5.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/386, 342, 391; 548/311.1, 317.1, 318.5, 320.1, 320.5.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,411,981 A (GAILLARD-KELLY ET AL.) 02 May 1995, examples 22, 23, 25, 31, 32, 43-46, 58, 65, 69-81, and 84-91 as well as column 46, line 55 to column 47, line 28.	1-16
X	EP 0,494,819 A1 (ROUSSEL-UCLAF) 15 JULY 1992, examples 22, 23, 25, 31, 32, and 43-46 as well as page 28, line 15 to page 29, line 8.	1-16
A	EP 0,436,426 B1 (ROUSSEL-UCLAF) 10 July 1991, entire document.	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 SEPTEMBER 1996

Date of mailing of the international search report
04 OCT 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
FLOYD D. HIGEL aco
Telephone No. (703) 308-1235

B. FIELDS SEARCHED
Documentation other than minimum documentation that are included in the fields searched:

Chemical Abstracts
Index Chemicus
Current Abstracts of Chemistry

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/415, C07D 233/84, 233/86, 233/72, 233/88, 405/04, 405/06

A1

(11) International Publication Number: **WO 97/00071**

(43) International Publication Date: 3 January 1997 (03.01.97)

(21) International Application Number: PCT/US96/10286

(22) International Filing Date: 13 June 1996 (13.06.96)

(30) Priority Data:
08/491,130 16 June 1995 (16.06.95) US

(71) Applicant *(for all designated States except US)*: BIOPHYSICA FOUNDATION [US/US]; 3333 North Torrey Pines Court #100, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SOVAK, Milos [DE/US]; 3333 North Torrey Pines Court #100, La Jolla, CA 92037 (US). BRESSI, Jerome, C. [US/US]; P.O. Box 126874, San Diego, CA 92112 (US). DOUGLASS, James, Gordon, III [US/US]; 4066 Moratalla Terrace, San Diego, CA 92130 (US). CAMPION, Brian [US/US]; 122 S. Rios Avenue #5, Solana Beach, CA 92075 (US). WRASIDLO, Wolfgang [US/US]; 307 Prospect Street, La Jolla, CA 92037 (US).

(74) Agents: ROWLAND, Bertram, I. et al.; Flehr, Hohbach, Test, Albritton & Herbert, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ANDROGENIC DIRECTED COMPOSITIONS

(57) Abstract

Substituted phenylthiohydantoins are provided for use in detecting the presence of tumor cells having androgenic receptors and providing for cytostatic and cytotoxic activity toward such cells. The subject compounds provide for vehicles for specific targeting to the endrogenic receptor containing cells of cytostatic and/or cytotoxic agents, heavy or light radioactive or radioopaque atoms, and the like for detection and treatment of cancer cells involving androgenic receptors or blocking androgenic receptors.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

ANDROGENIC DIRECTED COMPOSITIONS

INTRODUCTION

Technical Field

The field of this invention is diagnosis and treatment of androgenic related neoplasia and blockage of androgenic receptors.

Background

The growth of prostate cancer (CaP) depends upon the presence of androgen (male) hormones, acting via androgen receptors contained in the cell's nucleus. The only effective, albeit temporary, therapy of prostate cancer is based upon interference of male hormone production or activity, using estrogenic steroids or non-steroidal substances to block the cancer cells' androgen receptors. There are a number of problems with these therapies. Steroidal estrogens had to be abandoned due to their high cardiovascular toxicity. The only steroidal compound clinically used today is cyproterone acetate. However, it also binds to the glucocorticoid and progestin receptors. Current, clinically-used non-steroidal anti-androgens such as Flutamide, Casodex or Anandron do not bind sufficiently to androgen receptors to achieve their complete blockage. None of the current anti-androgens provide permanent relief. It is suspected that the incomplete blockage of the receptors may be the reason why, with time, the therapy invariably becomes ineffective as the CaP cells mutate having proliferated metastatically. At that phase, the cells cannot be substantially influenced by any known chemotherapy or radiation.

There is the further consideration that the current armamentarium for the diagnostic staging of prostate cancer is extremely poor and yet essential in choosing the therapeutic mode. Proof of metastatic dissemination beyond the prostate excludes surgery and relegates these patients to systemic therapy. With improved diagnostic staging, unnecessary prostatectomies, a major and potentially mutilating

surgery, could be avoided.

Only recently, an assay has become available for the detection of CaP cells circulating in the blood. However, that finding alone does not imply the existence of metastases. Typically, early metastases occur in the lymph nodes and the later ones develop in the bones. While ^{99}Tc scans can visualize bone defects, the lymph node metastases are extremely difficult to locate since typically, the infiltrated nodes are neither enlarged nor show changes on either magnetic resonance or x-ray computed tomography. Further, because of their low metabolic rate, the pathological nodes cannot be identified by positron emission spectrography using ^{18}F-deoxyglucose. Lymph node biopsy is possible only in the pelvic area. Early metastases in inaccessible paraaortic lymph nodes cannot be detected and consequently these patients are operated upon needlessly. Recently developed radiolabeled monoclonal antibodies against prostate cancer have only a limited use due to their low target specificity and long persistence in the blood pool, liver and spleen, which interferes with the imaging.

There have been a number of attempts to develop a CaP radionuclide scanning agent. Several radioiodinated androgen steroids were made, but they suffer from synthetic complexity. Steroidal androgens labeled with ^{18}F were synthesized as a potential PET imaging agent for prostate cancer, but their practicability is limited due to the complicated synthesis and need for specialized rare equipment (PET scanners) to detect positron emitting radionuclides. There is a further consideration that androgens promote CaP growth.

There is, therefore, substantial interest in developing novel compounds which can provide for the diagnosis and therapy of prostate cancer.

Relevant Literature

N-aryl substituted imidazolinediones have been reported in DE32 22 523; Offenlegungsschrift 26 49 925; WO88/03404; EP0 436426; EP0 494819; EP0 580459, and Teutsch, J. Steroid Biochem. Molec. Biol. (1994) 48:111-119. The activity of the trifluoromethyl, nitro- and trifluoromethyl, cyanophenyl derivatives as high-affinity ligands for the androgen receptor are reported in Teutsch, *supra.*, as well as in many of the foregoing patents.

SUMMARY OF THE INVENTION

Specific N-substituted 3-trifluoromethyl-4-cyano phenylthio-4',4'-dimethylhydantoins, their amino and thione analogs are provided having substitution at the remaining annular atom. Substituents include cyclic and aliphatic groups. Of particular interest are groups which can be used for imaging and/or have enhanced therapeutic index.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

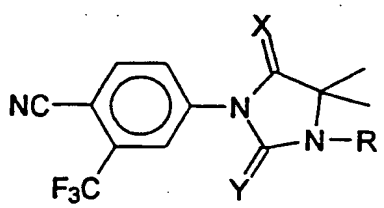
N-substituted arylthio-4',4'-dimethylhydantoins are provided, where when the 3'-N-substituent comprises other than an iodoaryl group, the hydantoin is a mono-thiohydantoin, where the other sp^2 carbon atom is bonded to oxygen, or nitrogen (imino). The compounds find use for diagnosis and/or therapy associated with androgenic receptors. The subject compounds have high affinity for androgen receptors of a variety of cell types and are able to exert at least one of proliferation inhibition or cytotoxicity for therapy or preferential binding for use as a detection medium for cells and tissues comprising androgenic receptors or for other identification.

For the most part, the subject compositions can be divided into three categories as characterized by the N-substituent: A group of from two to eight, usually from two to six carbon atoms, more usually from two to four carbon atoms, particularly two to three carbon atoms, which may be aliphatic or heterocyclic, generally having from zero to three, more usually from zero to two heteroatoms, preferably from one to two heteroatoms, which may be derivatized, particularly alkylated or acylated, where the alkyl or acyl group will be of from one to ten, more usually one to eight, preferably of from one to six carbon atoms, where the acyl group will generally be of from two to six carbon atoms, where the non-oxo-carbonyl may be bonded to from zero to two oxygen and/or nitrogen atoms, and zero to one carbon atoms; where the heterocycle will be from five to six annular members, particularly five annular members, where the annular members will be oxygen and nitrogen, generally having from 1 to 3 annular heteroatoms; the second group will have an agent, frequently a cytotoxic agent and/or imaging agent bonded to the hydantoin, normally through a linking group of from one to six, usually one to four carbon atoms, preferably two to three carbon atoms and one heteroatom,

where the linking group may include one or more functionalities, such as amino, oxy, and non-oxo-carbonyl, where amides and esters may be involved, e.g. urethanes; and the third group will involve carbocyclic aryl groups, particularly iodoaryl, which may be bonded to the nitrogen of the hydantoin through a linking group of from one to eight, usually two to six carbon atoms, preferably two to three carbon atoms, where the linking group may include an amino, oxy or non-oxo-carbonyl functionality, particularly ester or amide, and the aryl group may be substituted with oxy, amino, non-oxo-carbonyl, and derivatives thereof. As the aryl group, phenyl is of particular interest.

Tissue comprising cells with androgen receptors include prostate tissue, ovary tissue, testes, etc. Hosts of interest include primates, e.g. humans, domestic animals and pets.

The first group of the compounds of the subject invention will have the following formula:



wherein:

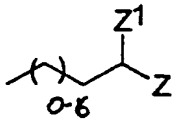
X is oxygen or nitrogen, with the proviso that when R is iodoaryl, X may be sulfur;

Y is sulphur, with the proviso that when R is iodoaryl group, Y may be sulphur, oxygen or nitrogen, preferably X and Y are different;

R is an organic group, which may be aliphatic and may comprise one or more heteroatoms, alicyclic, aromatic, heterocyclic, or combinations thereof, where heteroatoms include oxygen, nitrogen, sulphur etc., to be further defined below.

The first group of compounds will comprise monothiohydantoins, where the other oxo group of the hydantoin will be oxygen or nitrogen. These groups will, for

the most part, have R having the following formula:



wherein:

Z is hydroxyl, amino, a substituted amino or a 4-diazolyl, particularly a 4-(1',3'-imidazolyl);

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxolane.

The substituents on amino nitrogen may be varied widely, depending upon the use of the compound. For cytotoxicity or antiproliferative activity, the amino group may be unsubstituted or substituted, particularly with the single acyl group, where the acyl group may serve to enhance the activity of the compound by changing its pharmacokinetic activities, by providing for a second cytotoxic or antiproliferative compound, by providing for a chelating agent for chelating a metal ion, particularly a radioactive metal or non-metallic on, for carrying a radioopaque atom, or the like. Radioactive elements include fluorine, iodine, technetium, etc. Other metals of interest include gadolinium and the like.

Similarly, the hydroxyl, particularly the terminal hydroxyl, may be employed as a site for linking, forming ethers or esters, where the groups bound to oxygen will come within the above description; activations and displacement with other groups of interest, e.g. fluorine, sulphur alkyls and the like.

In addition, iodoaryl groups may be employed which are linked to the nitrogen through an alkyl chain, where the alkyl chain may be of from 1 to 6, usually from 1 to 4, preferably from 2 to 4 carbon atoms. The iodoaryl group may be linked directly to the carbon of the alkyl group or linked through a heteroatom, particularly nitrogen or oxygen, e.g. amide, secondary amine, ether, ester, etc. where the iodoaryl group may have a non-oxo-carbonyl or amino group linked to an annular carbon atom as part of the linking chain. The iodoaryl will generally have from 1 to 4, usually 2 to 4, more usually 2 to 3 iodine atoms, and may be further

substituted with oxy, particularly hydroxy or alkoxy of from 1 to 3 carbon atoms, or amino, or a substituted amino (mono- or disubstituted), having alkyl substituents having a total of 1 to 6 carbon atoms, more usually 1 to 4 carbon atoms, and 0 to n-1 oxy groups, where n is the number of carbon atoms in the substituent. A variety of aminosubstituted symmetrically substituted triiodoisophthaldiamides and diaminosubstituted symmetrically substituted triiodobenzamides have been reported in the literature, where the nitrogen atoms are substituted with acyl groups, alkyl groups or oxyalkyl groups of 1 to 6, usually 1 to 4 carbon atoms and 0 to n-1 oxy groups. See, for example, U.S. Patent Nos. 4,547,357; 4,021,481; 4,364,921 and 4,341,756 and references cited therein. The carboxyl group may be used to link the iodoaryl group to the thiohydantoin throgh the alkyl chain.Alternatively, iodine may be bonded to an sp_2 carbon atom of an alkenyl group.

Illustrative R groups include: allyl, propynyl, aminoethyl, aminopropyl, 2-hydroxypropyl, 3-hydroxypropyl, 2-hydroxyethyl, 2,3-dihydroxypropyl, 2-hydroxy-3-acetoxypropyl, 4-benzamidobutyl, 4-fluorobutyl, 4-iodobut-3-enyl, 2-iodoprop-2-enyl, cis & trans-3-iodo-prop-2-enyl, 3-(4'-oxazolyl-1,3)propyl, 2-(4'-diazolyl)ethyl, 3-(propionamido)propyl, N-phenoxycarbonyl 2-aminoethyl, N-methoxycarbonyl 2-aminoethyl, 3-(3',5'-diiodo-4'-dimethylaminophenyl)propyl, 2-(3',4',5'-triiodophenyl)propyl, N-(cysteinyl, glycyl, glycyl) 2-aminoethyl, (3',6',9'-triazanonoxy)ethyl, p-hydroxyphenylpropyl, and the carboxamide of N-nitrilotriacetic acid and 2-aminoethyl.

Alternatively, various cytotoxic agents may be employed, which are joined to the subject hydantoins by any convenient linking group, which does not significantly diminish the cytotoxic or antiproliferative activity of the compound. Compounds of interest include methotrexate, taxol, 5-fluorouracil, adriamycin, bleomycin, and the like.

The subject compounds can be prepared in accordance with conventional ways, varying the particular procedure based on the particular side groups. The preparation of hydantoins conveniently involves the use of an isocyanate and a substituted α-aminoacetonitrile. By appropriate choice of the isocyanate and the α-aminoacetonitrile, one may arrive at the final product in a single step. Alternatively, by employing various protective groups, which may be subsequently removed, or providing for substituents which become involved in the formation of

the hydantoin or may provide for sites for further derivatization. Various procedures are described in EPO Publication Numbers 0 494 819 and 0 580 459. Also, a significant number of examples may be found in the subject experimental section.

The subject compositions find a variety of uses associated with prophylactic and therapeutic opportunities. By providing for substituents which allow for detection by x-rays, molecular resonance imaging, radioactivity, or the like, regions of a mammalian host, particularly humans, can be investigated, where the regions are associated with an androgenic receptor. Thus, cells or tissues associated with the androgenic receptors may be visualized, so as to identify neoplasms, benign tumors, mobile cells, etc. Thus, by having substituents which have radioactive atoms, heavy metals, heavy atoms such as iodine, or the like, one can visualize physiological structures associated with androgenic receptors.

In addition, the subject compounds have proliferative inhibitory capability in inhibiting the proliferation of cells having androgenic receptors and dependent upon signal transduction associated with the androgenic receptors. The subject compounds are found to have a high affinity for the androgenic receptors, demonstrating enhanced activity as compared to prior substituted hydantoins.

In addition, the subject hydantoins can be used as vehicles for transporting other cytotoxic agents to the androgen receptor comprising cells. Thus, while at the same time inhibiting androgenic activation, other pathways which inhibit proliferation may also be addressed. Thus, one can greatly enhance the therapeutic index of a known chemotherapeutic agent by directing the chemotherapeutic agent to specific sites in the host.

The subject compositions may be formulated in accordance with conventional ways for use *in vivo*. The subject compounds are found to be stable in human plasma at physiological temperatures. The subject compounds are found to have substantially greater cytostatic and cytotoxic effects in inhibiting cell growth for neoplastic cells, as compared to normal cells, i.e. having a high therapeutic index. The subject compositions are readily formulated in conventional carriers, such as saline, phosphate buffered saline, vegetable oils, ethanol, or other physiologically acceptable carrier.

The concentrations used for the subject compounds in diagnosis and therapy

will be varied widely, depending upon the purpose of the compound, the patient being treated, the stage of the disease, whether the subject compounds are being used by themselves or in a combination therapy, the manner of administration, the responsiveness of the cancer cells to the drug, and the like. The particular dosage can be determined empirically. Other components of the formulation may include buffers, stabilizers, excipients, or the like. Depending upon the particular compound and its formulation, administration may be oral or parenteral, including intravascular, subcutaneous, intratumoral, intraperitoneally, etc.

The subject compounds may also be used in competitive assays for evaluating other compounds as to their cytotoxic or cytostatic effect. Thus, specific cell lines may be employed where the effect of an agent on the cytotoxic level of a subject compound may be determined in relation to the survival rate of the target cells. Also, in mixtures of cells containing neoplastic androgenic receptor containing cells the subject compounds can be used to eliminate the neoplastic cells in the presence of normal cells. Thus, in a variety of cultures, where androgenic receptor containing cells may be susceptible to becoming or are tumorous, by maintaining a cytoxic level of the subject compounds in the medium, the cells may be selectively killed.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

The following compounds were prepared according to the general method described by Teutsch et. al., J. Steroid Biochem. Molec. Biol. 1994; 1:11-119.

Example 1

4-[3′-(2″-(N-t-butoxycarbonyl)-aminoethyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-136)

Crude 2-trifluoromethyl-4-isothiocyanoato-benzonitrile (700 mg, 3.07 mmol) was dissolved in THF (6.0 mL). At room temperature, triethylamine (59μL, 0.42 mmol) was added to the stirring solution followed by 2-(1′,2′-ethyldiamino-N-t-butoxycarbonyl)-2-cyanopropane (682 mg, 3.00 mmol). The reaction was refluxed for 40 minutes under a N_2 atmosphere and then the solvent was removed under

reduced pressure. The resulting brown residue was purified by silica gel chromatography (CH_2Cl_2/acetone, gradient) and treated with activated carbon to yield 951 mg (68.1%) of light yellow powder.

mp: 81°C (dec); UV (MeOH):λ_{max}=234 nm (ϵ=18841) and 260 nm (ϵ=21454)

Example 2

4-[3′-(2″,2″-dimethyl-1″,3″-dioxolane-4″-methyl)-4′,4′-dime thyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-163)

BP-163 was prepared and purified as described in Example 1 using the amino cyanopropane prepared from 2,2-dimethyl-1,3-dioxolane-4-methanamine and acetone cyanohydrin. Yield = 63.3%.

UV (MeOH): λ_{max}=230 nm (ϵ=23528), 244 nm (ϵ=22733), and 258 nm (ϵ=24590);

Example 3

4-[3′-(2″-propenyl)-4′,4′-dimethyl-5′′-imino-2′-thioxo-1′′-imidazolidinyl]-2-trifluoromethy l-benzonitrile. (BP-208)

BP-208 was prepared and purified by the same method described in Example 1 using the amino cyanopropane prepared from allylamine and acetone cyanohydrin. Yield = 67.3%.

Example 4

4-[3′-(2″-propynyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-im idazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-211)

BP-211 was prepared as described in Example 1 using the amino cyanopropane prepared from propargyl amine and acetone cyanohydrin. The compound was purified by chromatography (CH_2Cl_2/acetone, 100% (50:50 gradient by 10% segments) and isolated as an orange oil. The product was not further characterized and was carried as is into the hydrolysis step. (Example 12)

Example 5

4-[3′-(2″-{4′″-imidazolyl}ethyl)-4′,4′-dimethyl-5′-imino-2′ -thioxo-1′-

imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-210)

BP-210 is prepared as described in Example 1 using the amino cyanopropane prepared from 4-(aminoethyl)imidazole and acetone cyanohydrin. The compound is purified by column chromatography and isolated as a pale yellow oil. It is used in the subsequent hydrolysis without further purification. (Example 13)

Example 6

4-[3′-(2″-p-hydroxyphenylethyl)-4′,4′-dimethyl-5′-imino-2′- thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-212)

BP-212 was prepared as described in Example 1 using the amino cyanopropane prepared from p-hydroxyphenethylamine and acetone cyanohydrin. Following silica gel chromatography (CH_2Cl_2)/acetone; gradient), a pale yellow solid was obtained, which was taken directly into the hydrolysis step without further characterization. (Example 14)

Example 7

4-[3′-(2″-aminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-138)

BP-136 (300 mg, 0.66 mmol) was dissolved in MeOH (3.5 mL) and 2N HCl (.065 mL) with stirring at room temperature. The reaction mixture was refluxed for two hours, then the solvent was removed under reduced pressure, and the resulting solid was crystallized as the hydrochloride from isopropanol. Yield 204 mg (79.0%).

mp: >200(C; UV (MeOH): δmax =234 nm (18441) and 252 nm (ϵ=20891)

Example 8

4-[3′-(2″,3″-dihydroxypropyl)-4′,4′-dimethyl-5′-oxo-2′-thio xo-1′′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-135)

BP-135 was prepared in the manner described in Example 7 using the appropriate imine (BP-163, example 2). The product was isolated by pouring the reaction mixture over a mixture of ice and water. The product was extracted with EtOAc, dried over MgSO4 and the solvent removed under reduced pressure. BP-

135 was purified by silica gel chromatography (CH2Cl2/ acetone; gradient) then treated with activated carbon to yield a hygroscopic amorphous solid. Yield = 68.1%.

UV (MeOH): λ_{max}=234 nm (ϵ=17480) and 254 nm (ϵ=19963);

Example 9

4-[3'-(2"-propenyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1''-imid azolidinyl]-2-trifluoromethyl-benzonitrile. (BP-82)

BP-82 was prepared in the same manner as described in Example 7 using the appropriate imine (BP-208, example 3). The product was isolated by pouring the reaction mixture over a mixture of ice and water. The product was extracted with EtOAc, dried over MgSO4 and the solvent removed under reduced pressure. BP-82 was purified by treatment with activated carbon and crystallization from isopropanol. Yield = 87.4%.

mp: 146-148°C; UV (MeOH):λ_{max}=232 nm (ϵ=18022) and 254 nm (ϵ=21877)

Example 10

4-[3'-(2"-N-(t-butoxycarbonyl)-aminoethyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-137)

BP-137 was prepared from BP-136 in the same manner as described in Example 7 except the reaction was heated at 50°C for eight hours. The resulting white crystalline precipitate was filtered off and washed with cold MeOH/H_2O (50:50). Yield = 87.1%.

mp: 173-175°C; UV (MeOH): λ_{max}=234 nm (ϵ=18573) and 256 nm (ϵ=21499)

Example 11

4-[3'-[2",2"-dimethyl-1",3"-dioxolane-4"-methyl)-4',4'-dime thyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benz onitrile. (BP-134)

BP-134 was isolated as an impurity in the silica gel chromatographic purification of BP-163.

mp: 50°C (dec); UV (MeOH): λ_{max}=234 nm (ϵ=18765) and

254 nm (ϵ=21499)

Example 12

4-[3′-(2″-propynyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-199)

BP-199 was prepared from the appropriate imine (BP-211, example 4) in the same manner as described in Example 7. The product was isolated as colorless crystals from CH_2Cl_2/hexane.

mp: 120-121°C (dec); UV:λ_{max}=206 nm (ϵ=17328), 232 nm (ϵ=18068), and 252 nm (ϵ=22003).

Example 13

4-[3′-(2″-{4″′-imidazolyl}ethyl)-4′,4′-dimethyl-5′-oxo-2′-t hioxo-1′-imidazolidinyl-2-trifluoromethyl-benzonitrile. (BP-213)

BP-213 was prepared from the appropriate imine (BP-210, example 5) in the same manner as described in example 7. The crude product was purified by column chromatography and isolated as a colorless solid in high purity ((96%, HPLC).

UV: λ_{max}=234 nm (ϵ=14113) and 254 nm (ϵ=1604).

Example 14

4-[3′-(2″-p-hydroxyphenylethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-214)

BP-214 is prepared from the corresponding imine (BP-212, example 6) in the same manner as described in Example 7. The crude product is crystallized from CH_2Cl_2/hexane as colorless crystals.

Example 15

4-[3′-(2″-N-acetylaminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-139)

The free amine of BP-138 (100 mg, 0.28 mmol) was dissolved in $(Ac)_2O$ (5.0 mL) and allowed to stir at room temperature for 30 minutes. The solvent was then removed under reduced pressure and the resulting off-white solid was purified by silica gel chromatography (CH_2Cl_2/acetone; 95:5) to yield 102 mg (91.6%) of

pure compound.

mp: 77-79°C (dec); UV (MeOH):λ_{max}=234 nm (ϵ=18694) and 254 nm (ϵ=21499)

Example 16

4-[3′-(2″-aminoethyl-N-(glycyl-N′″-(2′″-(triphenylmethylthioacetyl)-glycine)))-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-197)

Dicyclohexylcarbodiimide (DCC, 1.1 mg, 5.35 x 10-3 mmol) and the free base of BP-138 (1.9 mg; 5.35 x 10-3 mmol) were added to a stirring solution of N-[2-triphenylmethylthioacetyl)]-glycyl-glycine (2.0 mg, 4.46 x 10-3 mmol) in THF (0.200 mL) at room temperature. The reaction was heated at 35°C for two hours and then purified by preparative HPLC without further work-up. Yield = 50.2%.

Example 17

4-[3′-(4″-oxybutyl-O-glycyl-N′″-(2-(triphenylmethylthioacetyl)-glycine))-4′,4′-dimethyl- 5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-198)

To a stirred solution of N-[2-(triphenylmethylthioacetyl)]-glycyl-glycine (2.0 mg, 4.46 x 10-3 mmol) in THF (2.00 mL) was added DCC (1.1 mg, 5.35 x 10-3 mmol),4-[3'-(4"-hydroxybutyl)-4',4'-dimethyl-5'-oxo-2'-thioxo -1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile (RU 59063, 2.1 mg, 5.35 x 10-3 mmol) [Synthesized as described by Teutsch et. al., supra] and a crystal of DMAP. After stirring at room temperature for 45 minutes, the product was isolated by preparative HPLC. Yield = 56.8%.

Example 18

4-[3′-(2″-aminoethyl-N-(glycyl-N′″-(2-thioacetyl)-glycine) -4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-207)

Bu_3SiH is added to a stirring solution of BP-197 in 10% TFA/CH_2Cl_2 and is purified by preparative HPLC without further work-up. This product can now be used as a substrate for complexing with 99Tc by standard methods.

Example 19

4-[3′-(4″-oxybutyl-O-glycyl-N′″-(2-(thioacetyl)-glycine))- 4′,4′-dimethyl-5′-oxo-2′′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-209)

Bu_3SiH is added to a stirring solution of BP-198 in 10% TFA/CH_2Cl_2 and is purified by preparative HPLC without further work-up. This product can now be used as a substrate for complexing with ^{99}Tc by standard methods.

Example 20

4-[3′-trans-(2″-propenyl-3″-tributylstannyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-237)

BP-199 (1.05 g) was dissolved in dry toluene (100 mL) under N2. Bu3SnH (1.12 mL) and AIBN (68.5 mg) were added and the reaction mixture heated to reflux. After stirring for 24 hours, additional aliquots of Bu_3SnH (0.40 mL) and AIBN (10 mg) were added. After further stirring for 3 hours at reflux, the reaction was allowed to cool to room temperature and the volatiles removed under vacuum. The crude product was purified by column chromatog raphy and isolated as a pale oil (1.67 g).

HPLC analysis indicated the presence of two isomers.

Example 21

4-[3′-trans-(2″-propenyl-3″-*iodo)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl-2-trifluoromethyl-benzonitrile. (BP-305)

BP-237 is dissolved in a small amount of methanol. Radioiodination is accomplished using Na[^{125}I]I or Na[^{131}I]I or Na[^{123}I]I by known methods (see Hunter & Greenwood, Nature, 1962; 194:495-496]. TLC with autoradiography indicates 50-75% radiochemical yield.

Example 22

4-[3′-(4″-methanesulfonyloxybutyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-232)

RU-59063 (described by Teutsch *et al.*, supra and Example 17), was dissolved in methylene chloride, pyridine was added and the solution cooled to 0°C. Under N_2, methanesulfonic anhydride was added slowly and the reaction allowed to

warm to room temperature. The solution was cooled and pyridinium hydrochloride is filtered. The product was purified column chromatography (silica gel, $CHCl_3$/acetone; gradient 100% (85:15) and isolated as a colorless solid. m.p. 114-115°C.

Example 23

4-[3'-(4"-fluorobutyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-218)

a.) ^{19}F BP-218

RU 59063 (2.2 g) was placed in a 100 mL Schlenk flask with a stir bar and placed under N_2. Dry methylene chloride was added (15 mL) and the solution stirred under N_2 for 10 minutes. Pyridine (1.66 mL) was added, the solution cooled to -78°C with dry ice/acetone bath. Dimethyl aminosulphur trifluoride (DAST, 0.905 mL) was added dropwise and the reaction stirred at -78°C for 4 hours. The solution was then allowed to warm to room temperature and then taken to dryness. The product was isolated as a colorless oil by column chromatography (260 mg).

b.) ^{18}F BP-218

[^{18}F] Fluoride ion was produced by proton irradiation of oxygen-18 enriched (96% isotopic enrichment) held in an all-silver cyclotron target (330 μL target volume). The aqueous [^{18}F] fluoride was converted to a no-carrier added Kryptofix 2.2.2/K_2CO_3/^{18}F; prepared by addition of the [^{18}O] water/[^{18}F] solution to a mixture of the aminopolyether 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane (Kryptofix 2.2.2, 26.0 mg, 0.069 mmole) and potassium carbonate (2.3 mg, 0.0166 mmole) in a Vacutainer®. The vessel was placed in an oil bath at 110°C, and water was removed under a gentle stream of N_2, assisted by azeotropic distillation, each employing 0.5-0.8 mL CH_3CN.

The Kryptofix/K_2CO_3/^{18}F solution (1-50 mCi) in anhydrous acetonitrile (500 (L) was added to 2.0 mg of (4-[3'-(4"-methanesulfonyloxybutyl)-4'-4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl-2-trifluoromethyl-benzonitrile) (BP-232). The reaction mixture was heated for one hour at 110°C and then cooled before being injected onto a preparative HPLC system. The HPLC purification was performed on a C-18 reverse-phase preparative column and eluted with a 65:35 CH_3CN/H_2O solvent mixture (2 mL/min). Column effluent was monitored by a flow-through

radiation detector at 254 nm. The desired F-18 compound eluted at ~ 19 minutes. The solvents were evaporated in vacuo and the ^{18}F BP-218 was reformulated in saline.

Both radio-HPLC and radio-TLC were used to determine radiochemical purity. Purity by HPLC was determined using an ODS reverse-phase column, eluting with acetonitrile/water (80/20) with UV detection at 254 nm and a flow-through radiation detector. The retention time for F-18 BP-218 was 6.2 min.

Radio-TLC were performed as follows: silica gel plates; $CHCl_3$/acetone (95:5); F-18 BP-218 (Rf=0.5).

Example 24

7{5″,5″-dimethyl-4″-oxo-3″-[4″′-cyano-3′″-trifluoromethylphenyl-1′-imidazolidinyl]-2″-thioxo-1″-ethylcarbamoxy}paclitaxel. (BP-196)

A round bottom flask charged with paclitaxel (60 mg, 0.07 mmol), imidazole (90 mg, 1.32 mmol) and a magnetic stir bar was placed under a N_2 atmosphere. CH_2Cl_2 (1.5 mL) was added and the solution was stirred at room temperature. To the solution was added portionwise a solution of 1.0 M $ClSiEt_3$ in THF (5 x 100 μL, 0.5 mmol). The progress of the reaction was monitored by HPLC. Upon completion, the 2'-(triethylsiloxy)paclitaxel was purified by preparative HPLC yielding 51.3 mg (75%). Purity by HPLC 97%. Proton NMR of the product matched values given in the literature [Chandhary et. al., J. Org. Chem. 1993; 58(15):3798-3799]

A round bottom flask charged with 2'-(triethylsiloxy)paclitaxel (30 mg, 0.03 mmol) and p-nitrophenylchloroformate (310 mg, 1.50 mmol) and a magnetic stir bar was placed under a N_2 atmosphere. A solution of pyridine (200 μL, 0.247 mmol) in CH_3CN (1.0 mL) was added and the mixture stirred at room temperature for 30 minutes. The product 2′-(triethylsiloxy), 7-(p-nitrophenylcarbonoxy)paclitaxel was purified by preparative HPLC yielding 24.2 mg (69%). Purity by HPLC was 96%.

To a round bottom flask charged with 2′-(triethylsiloxy), 7-(p-nitrophenyl-carbonoxy)paclitaxel (28.0 mg, 0.014 mmol), 4-[3′-(2″-aminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile (2 X 8.0 mg, 0.44 mmol) and a magnetic stir bar was added CH_2Cl_2 (300 μL). The solution was stirred at room temperature for 4 hours and the product, 2′-(triethylsiloxy)-7{5″,5″-

dimethyl-4″-oxo-3″-[4′″-cyano -3′″-trifluoromethylphenyl-1′-imidazolidinyl]-2″-thioxo-1″-ethylcarbamoxy}paclitaxel, was purified by preparative HPLC yielding 8.2 mg (85%). Purity by HPLC 97%.

To a round bottom flask charged with 2′-(triethylsiloxy)-7{5″,5″-dimethyl-4″-oxo-3″-[4′″-cyano-3′″- trifluoromethylphenyl-1′-imidazolidinyl]-2″-thioxo-1″-ethylcarb amoxy}paclitaxel (5.0 mg, 0.004 mmol) and a stir bar was added formic acid (250μL). The solution was stirred at room temperature for 15 minutes and the volatiles removed under vacuum. BP-196 was purified by preparative HPLC yielding 4.6 mg (>99%). The purity by HPLC was 99%.

Example 25

4-[3′-(2″-{4″′-(2″′*iodo)imidazoyl}ethyl)-4′,4′-dimethyl-5′ -oxo-2′-thioxo-1′-imidazolidnyl]-2-trifluoromethyl-benzonitrile . (BP-216)

BP-213 is dissolved in methanol. Radioiodination is accomplished with chloramine-T and Na[^{125}I]I or Na [^{131}I]I or Na [^{123}I]I by standard methods [Hunter and Greenwood, Nature, 1962; 194: 495-496] The product is purified by HPLC.

Example 26

4-[3′-gem-(2″-propenyl-2″-tributylstannyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl)-2-trifluoromethyl-benzonitrile. (BP-300)

BP-199 (2.30 g) was placed in a three-neck 500 mL round bottom flask fitted with two rubber septa, an N_2 adapter and stir bar. Dry toluene was added (30 mL) followed by HSnBu3 (2.48 g). $Pd(PPh_3)_4$ (151 mg) was dissolved in toluene (30 mL) and added quickly to the previously prepared solution. After 24 hours of stirring at room temperature, an additional aliquot of $Pd(PPh_3)_4$ (50 mg) was added and the reaction heated at 65°C for 3 hours followed by stirring at room temperature for 48 hours. The reaction mixture was taken to dryness and the product(s) purified by column chromatography. HPLC analysis (C18 reverse phase, 75:25 ACN/H_2O) suggested that the major product was BP-300 and the minor product was BP-237 (79:21), based on NMR comparison of the corresponding iodo compounds. (Example 29)

Example 27

4-[3'-trans--(2"-propenyl-3"-iodo)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-305)

and

4-[3'-cis--(2"-propenyl-3"-iodo)-4',4'-dimethyl-5'-oxo-2'-t hioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-307)

BP-237 (82% pure, with the remainder the corresponding cis isomer BP-354, 370 mg) was dissolved in $CHCl_3$ (5 mL) and cooled to 0°C. In a separate flask I_2 was dissolved (146 mg) in $CHCl_3$ (15 mL) and added to the solution of BP-237. After 2 hours at room temperature, the volatiles were removed and the crude products separated and purified using column chromatography.

Example 28

4-[3'-(6"-methanesulfonyloxyhexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-328)

BP-327 (10.4g) was dissolved in methylene chloride (130 mL), pyridine (2.5 mL) was added and the solution was cooled to 0°C under N_2. Methanesulfonic anhydride (5.5 g) was dissolved in methylene chloride (100 mL) and the resulting clear solution added slowly to the former solution. After 30 minutes at 0°C, the solution was allowed to warm to room temperature at which time the volatiles were removed under vacuum. The crude product was dissolved in a minimum of chloroform, filtered, and purified using silica gel column chromatography. Combining the appropriate fractions followed by removal of volatiles gave the product as a light brown oil (8.8 g, 98% pure by HPLC).

Example 29

4-[3'-(6"-thiohexyl)hexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo- 1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-332)

BP-328 (1.10 g) was dissolved in methylene chloride (35 mL). In a separate flask were placed hexanethiol (315 μL) and toluene (10 mL). Sodium methoxide (403 μL, 5.5 M) was added and the solution stirred for ten minutes. The resulting emulsion was added dropwise to the BP-328 solution with rapid stirring. After stirring for 12 h, the solution was stripped down and the crude product was purified

by column chromatography and isolated as a clear oil (160 mg) in 25% recovered yield. Additionally, unreacted BP-328 was also recovered (50%).

Example 30

4-[3′-{2″-N-(p-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl benzonitrile. (BP-231)

A 100 mL Schlenk flask was charged with BP-138 (430 mg, 1.20 mmol), Bolton-Hunter reagent (318 mg, 1.20 mmol) and a stir bar. Anhydrous THF (5 mL) was added via a gas tight syringe and the reaction mixture stirred under N_2(g) at room temperature. After one hour, the volatiles were removed under vacuum and the crude product purified using column chromatography (230-400 mesh SiO_2, 20 g, packed with $CHCl_3$) using gradient elution (100% CHCl3 (80:20 $CHCl_3$/Acetone). The appropriate fractions (as determined by TLC) were combined and the volatiles removed to give the product as a white solid (385 mg) in 64% yield. The purity by HPLC was 99.0%. UV (MeOH): λ_{max}=206 nm (ϵ=9553), 228 nm (ϵ=9872), 254 nm (ϵ=8339).

Example 31

4-[3′-{2″-(N-3‴,5‴-diiodo-4‴-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl-benzonitrile. (BP-248)

BP-231 (54.2 mg, 0.107 mmol) and chloramine-T (60 mg) was placed in a round bottom flask and $CHCl_3$ (6 mL) added. Iodine was added (6.05 mg). Methanol (3 mL) was added dropwise at room temperature with stirring. The solution turned orange. After one hour, the reaction was quenched ($Na_2S_2O_5$ 50 mg in 5 mL H_2O) and the products extracted into $CHCl_3$ (2 x 10 mL). The combined organics were dried and the volatiles removed. The crude product was purified using column chromatography (SiO_2, 5 g, $CHCl_3$) with a gradient elution (100 $CHCl_3$ (95:5 $CHCl_3$/Acetone). Purity was 97% based on HPLC. Mass Spec: MH+ (757).

Example 32

4-[3'-(6"-hydroxyhexyl)-4',4'-dimethyl-5'-oxo-2'-thioxo-1'-imidazolidinyl]-2-trifluoromethyl-benzonitrile. (BP-327)

The amino cyanopropane derived from 6-hydroxyhexyl amine and acetone cyanohydrin (13.9 g, 75.7 mmol) was dissolved in THF (100 mL). In a separate flask was placed the substituted aryl isothiocyanate (17.2 g, 75.7 mmol) to which was added THF (50 mL) and NEt_3 (2.0 mL). The latter orange solution was added to the former with stirring. After 12 hours, the volatiles were removed under vacuum to give the crude imine cyclization product as an viscous orange oil. This product was dissolved in methanol (350 mL) and subjected to HCl (2N, 94 mL, 0.187 mmol). Heat evolved. After 30 minutes, the volatiles were removed under vacuum. The product was purified using column chromatography (250 g, SiO2, $CHCl_3$) and a gradient elution (100 CHCl3 (80:20 $CHCl_3$/Acetone). 26.0 g of product was obtained (light brown oil). Purity by HPLC: 98.8%.

Testing:

All compounds were tested for stability by incubation in human plasma at 38°C for three hours and subsequent analysis by high pressure liquid chromatography. All compounds tested were found to be stable under these conditions.

All compounds were screened on a panel of normal and cancer human cell lines, including human prostate cancer cell lines, PC-3, DU-145, and LnCAP. The purpose of this experiment was to assess cell growth inhibition by measuring cytotoxicity and cytostatic effects.

Cells (10^4/well) were plated on 96 well plates with the following controls: no cells and toxic control (1 x 10^{-3}M sodium dodecyl sulfate (SDS). The drug was diluted in ethanol and added directly to the wells. Plates were incubated at 37°C under 5% carbon dioxide in sterile air, in a humidified incubator for 72 hours. A solution (50 μl of 2,3-bis-(methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT), 1 mg/mL) in phosphate buffered saline (PBS, 100 mM) was added to each well, In the presence of viable cells, this colorless clear solution is enzymatically transformed to give a pink coloration, read at 450 nm using a microplate reader (Molecular Devices Thermomax). The inhibition of cell growth was measured by hemocytometer,

counting cell viability. (Table I)

The results of compounds hitherto investigated are shown in Tables I and II. While the cytostatic effect of BP-82 is demonstrated in PC-3 human cell line (Table II), the growth inhibition (which reflects primarily cytotoxicity and may obscure the cytostatic property) is shown for compounds BP-196 and BP-199.

It is not certain whether the cytoxicity of BP-196 can be ascribed to the taxol moiety. The toxicity of this compound vis-a-vis normal cells is also quite high.

On the other hand, it appears that such targeting does occur with BP-199 which is most cytotoxic in the human prostate cancer lines containing at least some androgen receptors, but has low cytotoxicity in a variety of other human transformed and normal cells.

The androgenic and anti-androgenic activity of the current and novel compounds was tested in a specific assay described by Fuhrman et al. [J. Steroid Biochem. Molec. Biol. 1992;42:787-793]. This assay uses CV-1 cells derived from monkeys transfected with human androgen receptors. (Table III and IV).

TABLE I

Inhibition of Cell Proliferation at 72 hours:
Cytotoxic Effects of the Selected Novel Anti-Androgens.

		IC_{50} [M]		
Cell Line	Tumor	BP-82	BP-196	BP-199
DU-145	Human Prostate (receptor poor)	1.39×10^{-5}	8.67×10^{-7}	8.51×10^{-5}
Ln CAP	Human Prostate (with androgen receptors)	6.60×10^{-5}	1.31×10^{-7}	8.20×10^{-7}
PC-3	Human Prostate (few androgen receptors)	3.15×10^{-5}	3.72×10^{-8}	1.32×10^{-7}
MCF-7	Human Breast	5.00×10^{-5}	9.89×10^{-7}	1.00×10^{-4}
MCF-7/ADR	Human Breast (adriamycin resistant)	1.51×10^{-5}	1.00×10^{-5}	1.00×10^{-5}
Ovcar 3	Human Ovary	9.65×10^{-5}	5.00×10^{-8}	$> 10^{-4}$
Molt-4	Human T-cell Leukemia	4.88×10^{-5}	1.47×10^{-7}	$> 10^{4}$
L-1210	Mouse Leukemia	2.50×10^{-5}	9.70×10^{-7}	1.10×10^{5}
	Normal			
NH DF	Dermal Fibroblast (human)	9.17×10^{-5}	1.07×10^{-7}	$>10^{-4}$
HLF-1	Normal Lung Diploid (human)	3.90×10^{-5}	8.06×10^{-6}	$>10^{-4}$
CHO	Chinese Hamster Ovary	3.45×10^{-5}	8.76×10^{-6}	1.28×10^{-5}

TABLE II

Relative Growth Inhibition*
Hydantoin Derivatives at 10^{-5} M after 6 days.

Compound	No. of cells remaining expressed as a % of control	Observation
BP-82	≈70%	growth reduction only
BP-196	≈100%	cytotoxic cell death
BP-199	≈50%	growth reduction only
BP-213	≈40%	some cytotoxicity and growth reduction
BP-231	≈30%	growth reduction only

*Cell density 10^4/well

TABLE III

Anti-androgenic potency (IC_{50}) of current and novel anti-androgens.

Transactivation assay in CV1-3.9.2 cells; Stimulation with 0.1 nM testosterone)

COMPOUND	IC_{50}[nM]
Cyproterone Acetate	11
RU59063†	23
Hydroxyflutamide	35 (Binding Affinity [Kf]*=280
Casodex	180
BP134	21
BP135	158
BP136	200
BP137	20
BP138	139
BP139	239
BP199	15 (Binding Affinity [Kf]*=5
BP82	≈ 6.5
BP163	217
BP 307	7 (Binding Affinity [Kf]*=24
BP 305	100 (Binding Affinity [Kf]*=15
BP 306	10 (Binding Affinity [Kf]*=23
BP 82	~6.5 (Binding Affinity [Kf]*=28
BP 231	260 (Binding Affinity [Kf]*=56
BP 328	NA (Binding Affinity [Kf]*=52
BP 218	NA
BP 332	NA

* Kf=competition factor, Kf=1=same as R1881
†Described by Teutsch, (Ref. 1)

TABLE IV

Androgen Activity of Anti-Androgens in CVI-3.9.2 Cells

Test Compounds*	CAT Activity [cpm]
EtOH+	2250
R1881 (0.1nM)+	5400
R1881 (1.0nM)+	5600
R1881 (10nM)+	6700
RU59063	2600
BP134	1600
BP135	1900
BP136	1800
BP137	2000
BP138	1600
BP139	1500
BP82	1300
BP163	2100

* (Except as indicated, all compounds were tested at 1 μM)
+ Controls

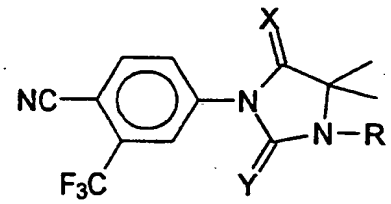
It is evident from the above results, that the subject compounds provide for a variety of advantages in directing a variety of agents to androgenic receptors of cells. Substantial therapeutic index is available between tumor cells and normal cells. The compounds are stable and can be readily formulated in a variety of ways. In addition, the subject compounds can be used as vehicles for bringing to tumor cells having androgenic receptors, cytotoxic agents, contrast agents, radioactive atoms, and the like. In this way, tumors having androgenic receptors may be visualized, as well as treated therapeutically.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound of the formula:



wherein:

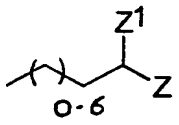
X is oxygen or nitrogen, where the proviso that when R is amino, oxy or iodo substituted aryl, X is sulfur, oxygen or nitrogen;

Y is sulphur, with the proviso that when R is said aryl group, Y is sulphur, oxygen or nitrogen;

R is an organic group comprising an aliphatic linking group of from 0 to 2 oxy groups, 0 to 1 amino group, 0 to 1 halo group, or 0 to 1 imidazolyl group, wherein said oxy groups, said amino group and said imidazolyl group have from 0 to 1 substituent.

2. A compound according to Claim 1, wherein R comprises an annular ring amino or oxy substituted aralkyl group for iodination or a polyiodoaralkyl group, wherein said aryl portion is linked to said alkyl portion by a carbon-carbon bond or through a heteroatom.

3. A compound according to Claim 1, wherein R is of the formula:



wherein:

Z is hydroxyl, amino, substituted amino, halo or 4-diazolyl;

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxalane.

4. A compound according to Claim 3, wherein Z and Z^1 are taken together.

5. A compound according to Claim 3, wherein Z is hydroxyl.

6. A compound according to Claim 3, wherein Z is amino.

7. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent being acyl or alkyl of from one to ten carbon atoms.

8. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent is a chelating group.

9. A compound according to Claim 3, wherein Z is monosubstituted amino, and said amino substituent is an antibiotic.

10. A compound according to Claim 9, wherein said antibiotic is paclitaxel.

11. A compound according to Claim 3, wherein Z is a substituted amino group, wherein the substituent of said substituted amino group is a polyiodoaryl group.

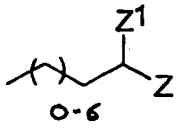
12. In a method for specifically directing an agent to cells comprising an androgenic receptor by adding said agent to a mammalian host comprising said cells, the improvement which comprises:

said agent being a compound according to Claim 1.

13. A method according to Claim 12, wherein said substituent is an antibiotic.

14. A method according to Claim 12, wherein said substituent comprises a radioactive atom or heavy atom.

15. A method according to Claim 1, wherein R is of the formula:



wherein:

Z is hydroxyl, amino, halo or 4-diazolyl;

Z^1 is hydrogen, hydroxyl, or may be taken together with Z to provide for olefinic or acetylenic unsaturation, or a 2,2-dimethyldioxalane.

16. A compound selected from the group consisting of: 4-[3′-(2″-propenyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′′-imid azolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-(N-t-butoxycarbonyl)-aminoethyl)-4′,4′-dimethyl-5′-imino-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-N-acetylaminoethyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-propynyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-trans-(2″-propenyl-3″-*iodo)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl-2-trifluoromethyl-benzonitrile; 4-[3′-cis--(2″-propenyl-3″-iodo)-4′,4′-dimethyl-5′-oxo-2′-t hioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(6″-thiohexyl)hexyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo- 1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(2″-{4″′-(2″′*iodo)imidazoyl}ethyl)-4′,4′-dimethyl-5′ -oxo-2′-thioxo-1′-imidazolidnyl]-2-trifluoromethyl-benzonitrile; 4-[3′-(4″-fluorobutyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-trans-(2″-propenyl-3″-tributylstannyl)-4′,4′-

dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile; 4-[3′-gem-(2″-propenyl-2″-tributylstannyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl)-2-trifluoromethyl-benzonitrile; 4-[3′-{2″-N-(p-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl benzonitrile; 4-[3′-{2″-(N-3‴′,5‴′-diiodo-4‴′-hydroxy phenethyl) amidoethyl}-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]- 2-trifluoromethyl-benzonitrile; and 4-[3′-(6″-methanesulfonyloxyhexyl)-4′,4′-dimethyl-5′-oxo-2′-thioxo-1′-imidazolidinyl]-2-trifluoromethyl-benzonitrile.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10286

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/415; C07D 233/84, 233/86, 233/72, 233/88, 405/04, 405/06.
US CL :514/386, 342, 391; 548/311.1, 317.1, 318.5, 320.1, 320.5.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/386, 342, 391; 548/311.1, 317.1, 318.5, 320.1, 320.5.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,411,981 A (GAILLARD-KELLY ET AL.) 02 May 1995, examples 22, 23, 25, 31, 32, 43-46, 58, 65, 69-81, and 84-91 as well as column 46, line 55 to column 47, line 28.	1-16
X	EP 0,494,819 A1 (ROUSSEL-UCLAF) 15 JULY 1992, examples 22, 23, 25, 31, 32, and 43-46 as well as page 28, line 15 to page 29, line 8.	1-16
A	EP 0,436,426 B1 (ROUSSEL-UCLAF) 10 July 1991, entire document.	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
04 SEPTEMBER 1996

Date of mailing of the international search report
04 OCT 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
FLOYD D. HIGEL aco
Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10286

B. FIELDS SEARCHED
Documentation other than minimum documentation that are included in the fields searched:

Chemical Abstracts
Index Chemicus
Current Abstracts of Chemistry